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Evaluation of New Rapid Commercial Enzyme Immunoassay for Detection of *Cryptosporidium* Oocysts in Untreated Stool Specimens

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Sixty-six stool specimens were evaluated by the ProSpecTR *Cryptosporidium* rapid enzyme immunoassay (EIA) (Alexon, Sunnyvale, Calif.). Approximately 2 g of untreated stool suspended in buffer was filtered through membranes labelled with anti-*Cryptosporidium*-specific antigen antibody. Anti-*Cryptosporidium*-specific antigen antibody was labelled with biotin, horseradish peroxidase conjugated to streptavidin, and tetramethylbenzidine, and each labelled antibody was added in sequence to the membranes. Each membrane had a positive control and test area. EIA results were compared with those of the modified acid-fast procedure. Twenty-three specimens were positive by the initial acid-fast procedure and the EIA. Forty-two specimens were negative by the initial acid-fast test and the EIA. One specimen was negative by the initial acid-fast test and positive by the EIA (sensitivity, 100%; specificity, 98.5%). This technique is easy to use by comparison with the cumbersome, labor-intensive, and more subjective microscopic methods currently available, and its sensitivity equals that of current microscopic methods.

Cryptosporidium spp. are coccidial protozoan (sporozoan) parasites which inhabit the intestinal and respiratory surface epithelium of mammals, birds, and reptiles and which cause the human clinical syndrome of cryptosporidiosis (11). *Cryptosporidium* spp. were first recognized in the gastric glands of laboratory mice by E. E. Tyzzer in 1907 (10). Between 1907 and 1955, *Cryptosporidium* spp. were described in many species of animals and were thought to be nonpathogenic (1). Over the next 20 years, cryptosporidial infection was associated with enterocolitis in various animals including turkeys, rattlesnakes, the European common fox, chickens, guinea pigs, sheep, rhesus monkeys, dogs, cats, cows, horses, pigs, deer, goats, gray squirrels, and raccoons (1, 2, 11). The first case of cryptosporidiosis in humans was diagnosed in 1976 in a 3-year-old child who exhibited symptoms of severe gastroenteritis for 2 weeks (1, 2). In the next 5 years, fewer than 12 cases of cryptosporidiosis were reported. In 1982, the Centers for Disease Control reported that 21 homosexual males with severe diarrhea had *Cryptosporidium* oocysts (1). Two prospective studies of patients with AIDS and diarrhea reported that 15% (National Institutes of Health) and 16% (Johns Hopkins Hospital) of the patients were found to have cryptosporidiosis (4, 7). In Haiti and parts of Africa, more than 50% of patients with AIDS were reported to be infected with *Cryptosporidium* spp. (8).

Cryptosporidial diarrhea has now been reported from six continents and in patients from 3 to 95 years old. Originally described as a zoonotic infection, cryptosporidiosis is now a well-recognized cause of diarrhea with abdominal pain in immunocompromised humans, immunocompetent children living in the tropics and the developed world, international travelers, and animal handlers (6).

A diagnosis of cryptosporidiosis is beneficial to both the patient and the physician in that it pinpoints the etiologic agent of infection and thus limits extensive further evaluation. In addition, an early and accurate diagnosis of cryptosporidiosis can reduce the use of empirical therapy for gastroenteritis which could be ineffective and potentially harmful (6). Ziehl-Neelsen staining is considered to be the best test for the detection of *Cryptosporidium* oocysts in stool specimens, especially in combination with a concentration method such as that involving Sheather's sugar (6).

Cryptosporidium-specific antigens (CSA) are associated with *Cryptosporidium* infections and are used as the basis for fluorescent and antigen capture immunoassays. ProSpecTR (Alexon, Sunnyvale, Calif.), a new rapid enzyme immunoassay (EIA), identifies the CSA that is produced by *Cryptosporidium* oocysts as they multiply within the intestinal tract of the host. This antigen has been found not to cross-react with those of other enteric parasites. CSA is derived from a protein extracted from the oocysts. The antigen is stable during transport through the intestinal tract of the host as well as during routine procedures used to collect and transport clinical specimens. The purpose of this study was to qualitatively and semiquantitatively evaluate a rapid (approximately 10 to 15 min), membrane-bound solid-phase immunoassay to detect CSA in aqueous extracts of fecal specimens by comparing it with the conventional microscopy method using the modified acid-fast stain.

Fresh, untreated stool specimens from 66 patients were tested within 48 h of collection. Cotton-tipped applicators were coated with approximately 2 g of the specimen, rotated several times in approximately 2 ml of specimen dilution buffer to suspend the fecal material in the solution, and then rolled firmly against the side of the vial to express as much of the fluid as possible. The consistency of the specimen determined the number of applicators used (two applicators for solid, four applicators for semisolid, and 6 applicators for liquid specimens). The specimen dilution was applied to reaction device membranes on which anti-CSA antibodies were immobilized. When it is present in a specimen, CSA bound the antibody on the membrane. After incubation, the membrane was washed to remove unbound material. An anti-CSA antibody labelled with biotin and one labelled with horseradish peroxidase conjugated to streptavidin were sequentially added, incubated, and washed. Tetramethylbenzidine was added, and the membranes

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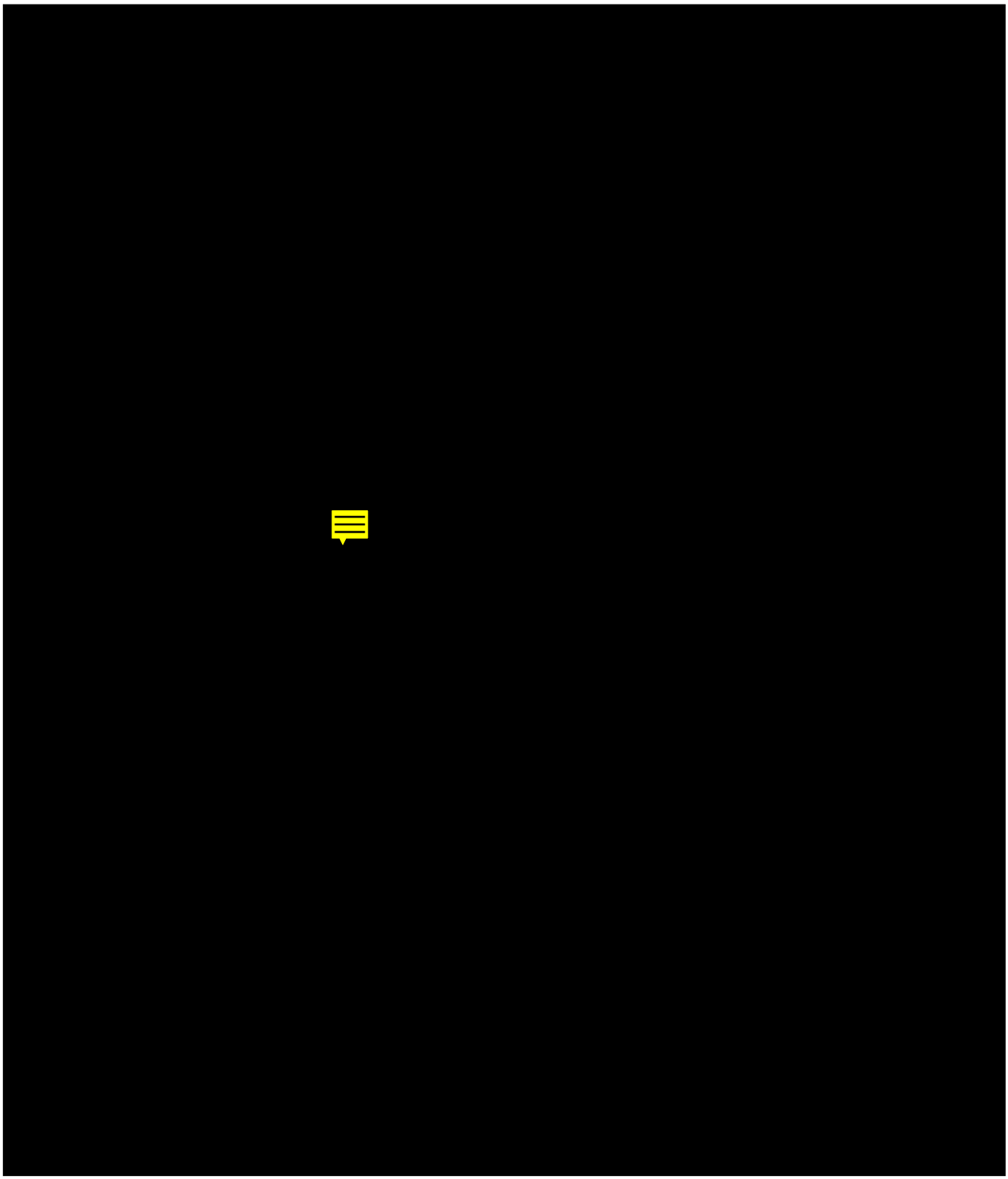

FIG. 1. (A) Schematic of a *Cryptosporidium* EIA positive reaction. TMB, tetramethylbenzidine; HRP, horseradish peroxidase; Ab, antibody. (B) *Cryptosporidium* rapid EIA results from positive (left) and negative (right) tests.

were examined after incubation. In a positive reaction, the binding of antibody-CSA-antibody-biotin-streptavidin-horseradish peroxidase-tetramethylbenzidine (Fig. 1A) resulted in a circular blue spot at the reaction site (Fig. 1B) which was detected visually. In a negative reaction, the lack of detectable CSA resulted in the absence of a circular blue spot or the formation of an arc. Each reaction device membrane contains a test area and a positive control (which contains CSA antigen

TABLE 1. Results of the modified acid-fast procedure compared with those of the *Cryptosporidium* rapid EIA

Cryptosporidium rapid EIA result	No. of specimens with the following acid-fast test result		Total no. of specimens
	Positive	Negative	
Positive	23	1	24
Negative	0	42	42
Total	23	43	66

and serves as a positive internal reagent and procedural control). Results are valid only if a positive reaction occurs at the control area.

Over a 4-month period, 23 stool specimens were consecutively positive by the modified acid-fast stain test and 43 stool specimens were consecutively negative by the modified acid-fast stain test. These results were compared with results obtained by the ProSpecTR *Cryptosporidium* rapid EIA. A second specimen from a patient with a previous positive was used only if the second specimen was collected more than 48 h from the time of the collection of the first specimen. The patient population included any patient of the New York University Medical Center who submitted a stool specimen to rule out *Cryptosporidium* infection. Positive modified acid-fast results were graded as few (one oocyst per most fields [magnification, ×950]), moderate (three to five oocysts), and many (more than five oocysts); negative modified acid-fast results were simply graded negative for oocysts. All results from the ProSpecTR *Cryptosporidium* rapid assay were valid (Table 1). Rapid assay results were graded as negative, weak, moderate, or strong. A negative test area with a positive control area represented a negative reaction. Positive test area results were semiquantitatively graded as follows: a faint blue represented weakly positive for CSA, a moderate blue represented moderately positive for CSA, and an intense blue represented strongly positive for CSA.

Qualitatively, all (100%) positive acid-fast specimens were positive by the *Cryptosporidium* EIA (Table 1). Semiquantitatively, the acid-fast results for 18 specimens (78%) correlated well with the *Cryptosporidium* EIA results (Table 2). The acid-fast results for five specimens (22%) varied slightly from the *Cryptosporidium* EIA results. Quantitative results may vary on the basis of the amount of specimen used (number of swabs), and this variability should be evaluated on the basis of the solidity of the stool. One specimen, which consisted of semisolid stool, was read as moderately positive by the acid-fast test. When the *Cryptosporidium* EIA was performed with two swabs of specimen, the results were weakly positive. In this case, the rapid assay was repeated with four swabs of specimen. The results were then characterized as moderately positive.

Qualitatively and semiquantitatively, 42 of 43 (98%) specimens which were negative by the acid-fast test were negative by the *Cryptosporidium* EIA (Table 1). All of these results were negative because of the complete absence of a blue spot. One (2%) negative acid-fast specimen was weakly positive by the *Cryptosporidium* EIA. Thus, the *Cryptosporidium* EIA appears to be more sensitive than the acid-fast test. A repeat acid-fast test performed on the specimen was also negative. This patient had a history of colitis, and numerous macrophages were present in the specimen. One possibility is that the CSA, which was probably present on the surface of the macrophages, was detected by the rapid assay although viable organisms, most likely, were absent. Nevertheless, for the sake of this study, this result was considered discordant.

TABLE 2. Quantitative results of positive and negative specimens

Type and no. of specimens	Result	
	Acid-fast test	Rapid EIA
Positive		
6	Few	Weak
7	Moderate	Moderate
5	Many	Strong
1	Many	Moderate
1	Many	Weak
1	Moderate	Strong
2	Moderate	Weak
Negative		
42	No oocysts	No reaction
1	No oocysts	Weakly positive

In conclusion, the *Cryptosporidium* rapid EIA was shown to have a sensitivity of 100% and a specificity of 98.5%. This rapid EIA is easy to use and is as sensitive as the cumbersome, labor-intensive, and more subjective microscopic methods currently available.

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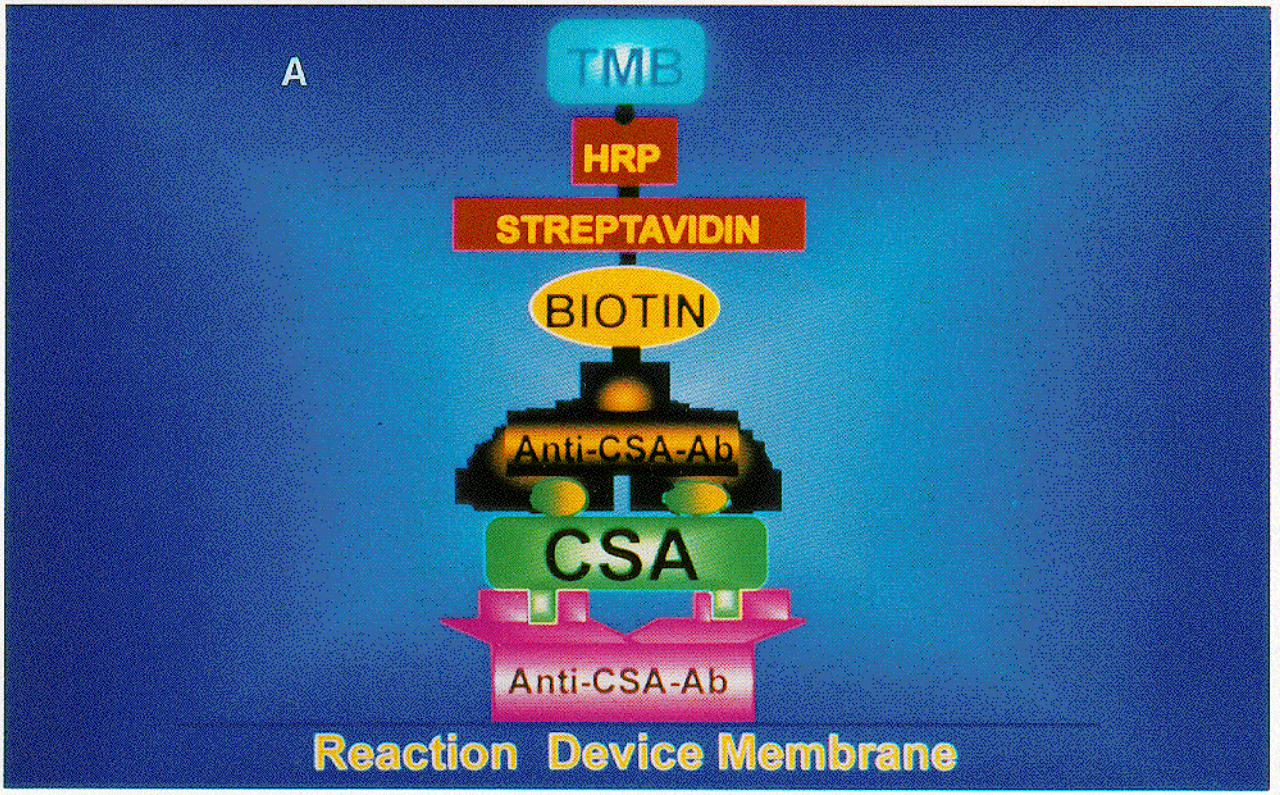
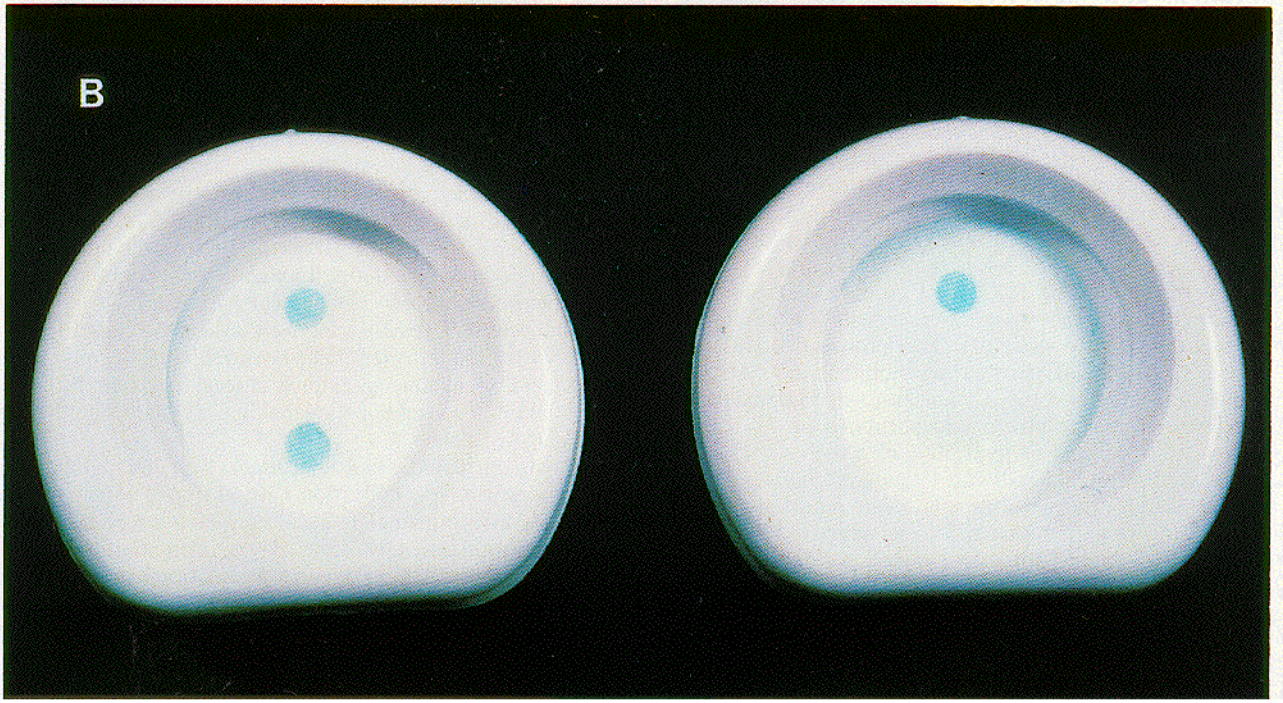

A
TMB
HRP
STREPTAVIDIN
BIOTIN
Anti-CSA-Ab
CSA
Anti-CSA-Ab
Reaction Device Membrane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